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First "hybrid" ligands of vanilloid TRPV1 and cannabinoid CB_2 receptors and non-polyunsaturated fatty acid-derived CB_2-selective ligands

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Abstract 12-Phenylacetyl-ricinoleoyl-vanillamide (phenylacetylrinvanil, PhAR, IDN5890), is an ultra-potent agonist of human vanilloid TRPV1 receptors also endowed with moderate affinity for human cannabinoid CB_2 receptors. To improve its CB_2 affinity and temper its potency at TRPV1, the modification of the polar headgroup and the lipophilic 12-acylgroup of PhAR was pursued. Replacement of the vanillyl headgroup of PhAR with various aromatic or alkyl amino groups decreased activity at TRPV1 receptors, although the dopamine, cyclopropylamine, 1′-(*R*)- and 1′-(*S*)-methyl-ethanolamine, and ethanolamine derivatives retained significant potency (EC_{50} 31–126 nM). Within these compounds, the 12-phenylacetylricinoleyl cyclopropylamide and ethanolamide were the strongest ligands at CB_2 receptors, with K_i of 22 and 44 nM, and 14- and >20-fold selectivity over cannabinoid CB_1 receptors, respectively. The propyl- and allyl-derivatives also exhibited high affinity at CB_2 receptors (K_i = 40 and 22 nM, with 40 and >80-fold selectivity over CB_1 receptors, respectively), but no activity at TRPV1 receptors. The cyclopropyl- and allyl-derivatives behaved as CB_2 inverse agonists in the GTP-γ-S binding assay. Addition of *para*-methoxy, *-tert*-butyl or -chlorine groups to the 12-phenylacetyl moiety of PhAR produced compounds that retained full potency at TRPV1 receptors, but with improved selectivity over CB_2 or CB_1 receptors. Thus, the manipulation of PhAR led to the development of the first CB_2/TRPV1 dual ligands and of an entirely new class of inverse agonists at CB_2 receptors. Both types of compounds might find application in the treatment of inflammation, and represent new molecular probes to investigate the endocannabinoid–endovanilloid signalling system.

Keywords: Endocannabinoid; Vanilloid; Inflammation; Receptor; Channel; Anandamide

1. Introduction

Recent studies have highlighted the importance of "multiple-target" therapies for the treatment of chronic and inflammatory pain and migraine [1–4]. Surprisingly, while drugs with a pleiotropic mechanism of action are under development as anti-tumor agents [5], the therapeutic potential of compounds with more than one target in nociception is still largely unexplored. Over the past few years, two types of receptors have gained growing relevance as molecular targets for the discovery of new analgesic and anti-inflammatory drugs, namely the cannabinoid receptors subtype 1 and 2 (CB_1 and CB_2), and the transient receptor potential channels of type V1 (TRPV1, also known as vanilloid VR1 receptors). These receptors were identified in the course of studies of the molecular mode of action of two plant natural products, the psychotropic principle of *Cannabis*, (−)-Δ^9-tetrahydrocannabinol, and the pungent component of hot chilli peppers, capsaicin [6–8]. An opposing role for CB_1/CB_2 and TRPV1 receptors in the tonic control of nociception and as molecular transducers of inflammatory and thermal pain, respectively, has been demonstrated (see [9,10] for reviews). Accordingly, agonists of both cannabinoid receptor subtypes (see [9] for review), as well as agonists and antagonists of TRPV1 channels produce strong antinociceptive effects in animal models of chronic, neuropathic and inflammatory pain (see [10] for review). In particular, the analgesic and anti-inflammatory effects of TRPV1 agonists appear to be paradoxical as TRPV1 receptors are coupled to the release of pro-algesic and pro-inflammatory mediators from sensory neuron efferents. However, TRPV1 is immediately desensitized after its activation by agonists, thus rendering these neurons refractory to further stimulation, with subsequent analgesic and anti-inflammatory effects being observed after a first pro-nociceptive response to the agents [10]. Also "indirect" agonists of cannabinoid receptors, i.e. inhibitors of endogenous cannabinoid (endocannabinoid) inactivation, have been suggested as possible leads for the development of analgesic drugs [11,12]. Finally, recent evidence also points to cannabinoid CB_2 receptor antagonists/inverse agonists as promising anti-inflammatory agents [13,14].

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It has been observed that successful analgesic and anti-inflammatory agents, once assumed to function via a single mechanism of action, can instead interact with several molecular targets. Thus, it was shown that some cyclooxygenase inhibitors also inhibit endocannabinoid hydrolysis via the fatty acid amide hydrolase (FAAH) [12,15], or bind with moderate affinity to cannabinoid CB_2 receptors [15]. Furthermore, acetaminophen, possibly the most popular analgesic drug, may act in vivo partly through its conversion into AM404 [16], a compound that inhibits endocannabinoid cellular reuptake and activates/desensitizes TRPV1 receptors [17–19]. We have previously shown that "hybrid" agonists of CB_1 and TRPV1 receptors, like arvanil [20–23] and O-1861 [24], produce stronger analgesic actions than those observed with "pure" agonists with the same affinity for each receptor type. In particular, arvanil inhibits both the first and, particularly, the second phase of formalin-induced nocifensive behaviour [23], as well as visceral nociception in the mouse *p*-phenylquinone test [25]. Despite: (1) these promising results with CB_1/TRPV1 "hybrid" ligands; (2) the well-established role of CB_2 receptors in inflammatory and neuropathic conditions [26,27]; and (3) the growing evidence that some COX inhibitors may act in part via CB_2 or TRPV1 receptors [12,15], no CB_2/TRPV1 "hybrid" ligand has been developed to date. We recently reported that phenylacetylrinvanil (PhAR, IDN5890), a compound obtained by implanting a structural element of resiniferatoxin (RTX) on the capsaicinoid olvanil, is a very potent and efficacious agonist of human vanilloid TRPV1 receptors, with moderate affinity for human cannabinoid CB_2 receptors (Fig. 1) [28]. To temper its strong potency at TRPV1, a property that is useful to treat bladder hyperreactivity but may cause undesirable side effects in vivo, and to improve its affinity for CB_2, we have modified here the polar headgroup of phenylacetylrinvanil and its lipophilic 12-acyl moiety, obtaining not only the first class of "hybrid" dual ligands of CB_2 and TRPV1 receptors, but also the first examples of fatty-acid derived selective CB_2 ligands lacking polyunsaturation. Both types of compounds are of potential interest for the treatment of inflammation, and represent new molecular probes to investigate the endocannabinoid–endovanilloid system.

2. Materials and methods

2.1. Synthesis and characterization of compounds

12-Acyl derivatives of ricinoleic acid were obtained by esterification of the trichloroethyl ester of ricinoleic acid with the suitable acid and deprotection, and their amidation was carried out with the PPAA-TEA protocol [29] (Fig. 1). All end products (Table 1) were thoroughly characterized spectroscopically (^{1}H- and ^{13}C NMR, IR, HR-MS).

2.2. Binding assays for affinity at human recombinant cannabinoid receptors

For CB_1 and CB_2 receptor binding assays, the new compounds were analyzed by using P_2 membranes from COS cells transfected stably with either the human CB_1 or CB_2 receptor and [^{3}H]-(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxy-propyl)-cyclohexanol ([^{3}H]CP-55,940) as the high-affinity ligand as described by the manufacturer's instructions (Perkin Elmer, Italia). Displacement curves were generated by incubating drugs with 0.5 nM of [^{3}H]CP-55,940. In all cases, K_i values were calculated by applying the Cheng–Prusoff equation to the IC_{50} values (obtained by GraphPad) for the displacement of the bound radioligand by increasing concentrations of the test compounds.

2.3. GTP-γ-S binding assays

These assays were performed as previously described [30], using CHO cells stably overexpressing the human recombinant CB_2 receptor (10 μg/ml protein), 0.7 nM [^{35}S]GTP-γ-S and 320 μM GDP, and a final assay volume of 250 μl.

2.4. Assays for activity at human recombinant TRPV1

Human embryonic kidney (HEK) 293 stably overexpressing recombinant human TRPV1 cDNA were grown as monolayers in minimum essential medium supplemented with non-essential amino acids, 10% fetal calf serum, and 0.2 mM glutamine, and maintained under 95%/5%O_2/CO_2 at 37 °C. The effect of the substances on $[Ca^{2+}]_I$ was determined by using Fluo-3, a selective intracellular fluorescent probe for Ca^{2+}. One day prior to experiments, cells were transferred into six-well dishes coated with Poly-L-lysine (Sigma) and grown in the culture medium mentioned above. On the day of the experiment, the cells (50–60 000 per well) were loaded for 2 h at 25 °C with 4 μM Fluo-3 methylester (Molecular probles) in DMSO. After the loading, cells were washed with Tyrode pH = 7.4, trypsinized, resuspended in Tyrode, and transferred into the cuvette of the fluorescence detector (Perkin–Elmer LS50B) under continuous stirring. Experiments were carried out by measuring cell fluorescence at 25 °C (λ_{EX} = 488 nm, λ_{EM} = 540 nm) before and after the addition of the test compounds at various concentrations. Potency data are expressed as the concentration exerting a half-maximal effect (EC_{50}), whereas the efficacy of the effect was expressed as percent of the maximal observable effect produced by ionomycin (4 μM) (Fig. 2).

Affinity for TRPV1 was assessed by means of binding assays carried out as described previously [20] with membranes from HEK cells stably overexpressing the human recombinant TRPV1, and using [^{3}H]resiniferatoxin ([^{3}H]RTX) as the high-affinity ligand and RTX (1 μM) to calculate specific binding (62 ± 5% of total binding).

2.5. Anandamide hydrolysis assays

The effect of compounds on the enzymatic hydrolysis of [^{14}C]anandamide (6 μM) was studied by using membranes prepared from rat brain incubated with increasing concentrations of compounds in 50 mM Tris–HCl, pH 9, for 30 min at 37 °C. These conditions are optimal for FAAH, the enzyme that catalyzes the hydrolysis of both anandamide and 2-arachidonoylglycerol. [^{14}C]Ethanolamine produced from [^{14}C]anandamide hydrolysis was measured by scintillation counting of the aqueous phase after extraction of the incubation mixture with 2 volumes of $CHCl_3$/CH_3OH (2:1 by volume).

3. Results

3.1. Activity of the novel compounds at human recombinant cannabinoid CB_1 and CB_2 receptors

Table 1 shows the affinity constants of the novel compounds for human recombinant cannabinoid CB_1 and CB_2 receptors as compared to the parent compound, PhAR. All compounds with a non-phenolic headgroup exhibited higher affinity at CB_2 receptors than PhAR, while the serotonin, dopamine and tyramine derivatives (compounds **4**, **2** and **3**, respectively) showed only weak activity at both cannabinoid receptor types. All compounds with significant affinity for CB_2 were from 5- to 80-fold selective vs. CB_1 receptors. The most potent CB_2 ligands were the amides with ethanolamine (**5**), propylamine (**9**), allylamine (**10**), cyclopropylamine (**12**) and 2′-chloro-ethanolamine (**13**), with K_i values ranging between 22 and 44 nM. Modification of the 12-phenylacetyl moiety of PhAR led to compounds with decreased affinity for CB_2 receptors, and, as with PhAR, only very weak CB_1 affinity.

Two of the most potent ligands of CB_2 receptors found here, i.e. the allyl and cyclopropyl PhAR derivatives (compounds **10** and **12**, respectively), behaved as inverse agonists in the GTP-γ-S binding assays (Fig. 3A and B). They inhibited [^{35}S]GTP-γ-S binding (and hence G-protein-coupled receptor activity) to

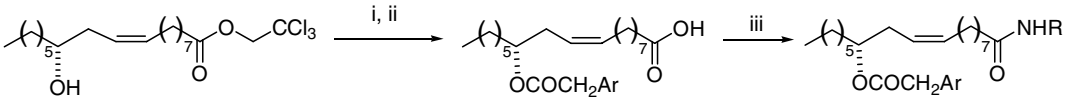


Fig. 1. General synthetic scheme for the novel compounds reported here. Legend: i: $ArCH_2COOH$, DCC, DMAP; ii: Zn, HOAc (overall 30–50%); iii: PPAA, TEA, RNH_2(20–70%) DCC = dicyclohexylcarbodiimide; PPAA = propylphosphonic acid anhydride; TEA = triethylamine.

membranes from cells stably overexpressing the human CB_2 receptor, with potencies (59.1 and 12.8 nM, respectively) not different from their K_i values in CB_2 binding assays (see Table 1). Compound **10** was less potent but more efficacious than the well established CB_2 inverse agonist/antagonist SR144528, whereas compound **12** was as potent and efficacious as SR144528 (Fig. 3C).

3.2. Activity of the novel compounds at human recombinant vanilloid TRPV1 receptors

Table 1 and Fig. 2 show the EC_{50} values and the TRPV1-mediated elevation by the novel compounds of intracellular Ca^{2+} concentrations in HEK-293 overexpressing the human recombinant TRPV1, as compared to the parent compound, PhAR. The introduction of various substituents at the *para*-position of the phenylacetyl moiety was tolerated, but replacement of the vanillamine headgroup with other phenolic amines as well as with various aliphatic aminoalcohols and aliphatic amines caused, to a various extent, an overall decrease of activity. Thus, the three novel 12-acyl analogues of PhAR (compounds **14**–**16**) were as potent as, but less efficacious than, the parent compound, while the twelve compounds with a non-vanillyl polar headgroup exhibited decreased TRPV1 potency. This decrease was less marked for the dopamide (**2**), followed by the cyclopropylamide (**12**), 1′-(*R*)- and 1′-(*S*)-methyl-ethanolamides (**7** and **6**), and ethanolamide (**5**) derivatives (EC_{50} ranging between 31 and 126 nM). Remarkably, the ethanolamide (**5**) was also the most efficacious non-vanillyl TRPV1 agonist in the present study, whereas the other non-vanillyl compounds exhibited a significantly lower efficacy than PhAR and capsaicin [24]. No compound exerted any effect on $[Ca^{2+}]_I$ in wild-type HEK-293 cells (not shown). Finally, the two most efficacious TRPV1 agonists (compounds **2** and **5**) were also assayed in binding assays with [^{3}H]RTX. In agreement with previous studies with capsaicin, arvanil and other fatty acid amide derivatives [20], the two compounds exhibited K_i values significantly higher than their EC_{50} values in the Ca^{2+} assays (Table 1).

3.3. Activity of the novel compounds on anandamide hydrolysis

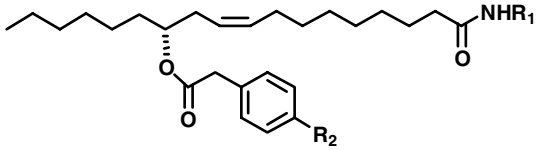
None of the 15 novel compounds exhibited any inhibitory action against [^{14}C]anandamide hydrolysis by rat brain membranes up to a 50 μM concentration (data not shown).

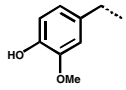
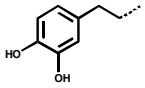
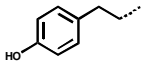
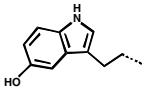
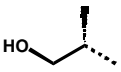
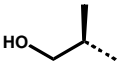
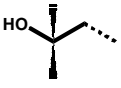
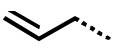
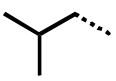
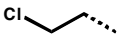
4. Discussion

We have reported here the discovery of the first "hybrid" cannabinoid CB_2 and vanilloid TRPV1 receptors ligands. We had previously developed "hybrid" cannabinoid CB_1/TRPV1 ligands, exemplified by arvanil [20–23] and O-1861 [24]. When given systemically, these compounds showed potent analgesic properties, and arvanil was also found to counteract cancer cell proliferation in vitro [21], neuronal excitotoxicity [31], spasticity in multiple sclerosis [23], and hyperkinesia in Huntington's disease [32] in vivo. On the other hand, because of their partial agonist activity at CB_1 receptors and their very high potency at TRPV1 receptors, arvanil and O-1861 also exert profound central cannabimimetic actions (including hypothermia, hypolocomotion and catalepsy [20,24,33]) when administered systemically in rodents, and burning pain when given topically [34]. These effects might limit the therapeutic use of these compounds, as well as of analogs showing a similar pattern of TRPV1-CB_1 activation. On the other hand, compounds that at the same time behave as inverse agonists at CB_2 receptors and agonists at TRPV1 receptors, particularly when not too potent as vanilloid agonists, are expected to be devoid of most of the undesired effects exhibited by arvanil and O-1816, and to maintain efficacy as anti-inflammatory agents. We have now identified two compounds endowed with these pharmacological properties, both obtained by chemical modification of PhAR [28], a compound showing ultra-potent activity at TRPV1 channels but weak affinity at CB_2 receptors.

It was previously shown that the acylation of arachidonic acid with ethanolamine, propylamine, 1′(*R*)-methyl-ethanolamine and, particularly, cyclopropylamine and 2′-choloroethanolamine result in ligands with high affinity for CB_1 receptors and ~10 to ~2000-fold selectivity over CB_2 receptors [35–37]. Surprisingly, when we replaced the vanillamine moiety of PhAR with these headgroups, the affinity for CB_2 receptors increased more dramatically than for CB_1 receptors. Within the generally low CB_1-affinity observed for these compounds, the cyclopropylamide (**12**) and 2′-choloroethanolamide (**13**) of 12-phenylacetylricinoleic acid did show the highest affinity for CB_1, in agreement with data with the corresponding arachidonic acid amides [36]. Nevertheless, a significantly higher affinity for CB_2 receptors was observed, with a ~14- and ~10-fold selectivity over CB_1 receptors, respectively. Even more strikingly, the propyl- and allyl-amides (compounds **9** and **10**) possessed a ~40- and ~80-fold selectivity over CB_1 receptors, respectively. These compounds, with K_i values of 40 and 22 nM, respectively, represent an entirely new class of high-affinity CB_2 ligands, developed for the first time from a long chain fatty acid amide lacking polyunsaturation. These surprising findings suggest that these five headgroups cause a switch in selectivity from CB_1 to CB_2 receptors when they are acylated with a fatty acid different from arachidonic acid, and whose polyunsaturation is replaced by the presence of a lipophilic aryl-containing group. Within these five amides, however, only the cyclopropylamide (**12**) and, especially, the ethanolamide (**5**) of 12-phenylacetylricinoleic acid retained some of the high potency and efficacy of PhAR at TRPV1 receptors, thus behaving as true dual CB_2/TRPV1 ligands. In agreement with previous structure–activity studies carried out on TRPV1 and cannabinoid receptors, the vanillylamine (**1**) and, to some extent, the catecholamine "heads" (**2**), but not the serotonin (**4**) and tyramine (**3**) groups, yielded the most potent and efficacious compounds at TRPV1, but not CB_2,

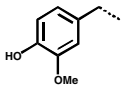
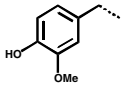
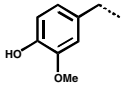
Table 1
General chemical structure of the compounds described here and their functional activity at human TRPV1 (expressed as potency, in EC_{50}, nM, and efficacy as percent of the effect of 4 μM ionomycin), and affinity for human CB_1 and CB_2 receptors (expressed as K_i, μM)



Compound number	R_1	R_2	hTRPV1			hCB_1	hCB_2
			K_i (nM)	EC_{50} (nM)	Max (%)	K_i (μM)	K_i (μM)
1		H	–	0.09 ± 0.01	95.0 ± 2.0	2.2 ± 0.2	0.3 ± 0.05
2		H	90 ± 5	31 ± 2	83.2 ± 4.0	1.25 ± 0.1	>5
3		H	–	ND	4.4 ± 1.5	>5	>5
4		H	–	ND	3.0 ± 1.0	>5	>5
5		H	227 ± 25	105 ± 5	73.0 ± 3.5	0.9 ± 0.05	0.044 ± 0.003
6		H	–	126 ± 8	16.9 ± 4.5	5.0 ± 0.5	0.54 ± 0.05
7		H	–	63 ± 3	20.2 ± 3.5	1.75 ± 0.2	0.20 ± 0.03
8		H	–	ND	5.2 ± 2.5	>5	0.27 ± 0.03
9		H	–	ND	3.3 ± 2.3	1.6 ± 0.2	0.040 ± 0.004
10		H	–	ND	5.4 ± 2.7	1.75 ± 0.3	0.022 ± 0.002
11		H	–	ND	1.3 ± 0.8	>5	0.124 ± 0.02
12		H	–	63 ± 4	28.9 ± 2.3	0.31 ± 0.04	0.022 ± 0.003
13		H	–	ND	8.8 ± 3.7	0.36 ± 0.05	0.040 ± 0.005

(*continued on next page*)

Table 1 (*continued*)

Compound number	R_1	R_2	hTRPV1			hCB_1	hCB_2
			K_i (nM)	EC_{50} (nM)	Max (%)	K_i (μM)	K_i (μM)
14		OCH_3	–	0.15 ± 0.02	59.6 ± 3.3	1.28 ± 0.1	2.6 ± 0.3
15		*tert*-butyl	–	0.35 ± 0.04	61.5 ± 3.9	> 5	> 5
16		Cl	–	0.19 ± 0.03	61.8 ± 4.1	1.30 ± 0.2	0.79 ± 0.11

Affinity for human TRPV1 receptors was measured by displacement binding assays against ^{3}[H]RTX and only for the two most efficacious TRPV1 agonists. Data are means ± S.D. of $N = 3$ experiments.

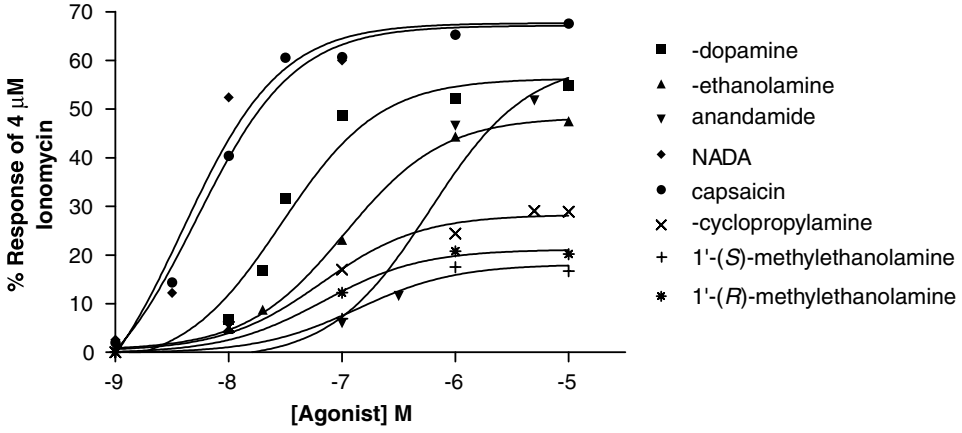


Fig. 2. Dose–response curve for the effects on $[Ca^{2+}]_I$ in HEK-293 cells overexpressing the human recombinant TRPV1 receptor, of: (1) the amides of 12-phenylacetylricinoleic acid with ethanolamine (**5**), dopamine (**2**), cyclopropylamine (**12**), 1′-(*R*)- methyl-ethanolamine (**7**) and 1′-(*S*)-methyl-ethanolamine (**6**); (2) *N*-arachidonoyl-ethanolamine (anandamide); (3) *N*-arachidonoyldopamine (NADA) and (4) capsaicin. Data are means of $N = 3$ experiments. Standard deviation bars are not shown for the sake of clarity and were never higher than 10% of the means. No significant effect was observed with any of the substances in wild-type HEK-293 cells.

receptors. Finally, amidation with 2-hydroxy-2-methyl ethylamine (**8**) or 1′(*S*)-methyl-ethanolamine (**6**) yielded weak CB_2 and TRPV1 ligands, almost inactive at CB_1 receptors, again in agreement with previous studies [37]. None of the new compounds could inhibit anandamide hydrolysis catalyzed by FAAH-containing rat brain membranes, thus suggesting that these compounds are not substrates for this enzyme and hence more metabolically stable than the corresponding arachidonic acid derivatives.

Although the presence of a 12-phenylacetylricinoleoyl group instead of a C20:4 acyl group in allyl-, cyclopropyl- and hydroxy-ethyl amides produces an interesting shift from high CB_1 to high CB_2 affinity, the most potent CB_2 ligands found here, i.e. the allyl- and cyclopropylamides of PhAR (compounds **10** and **12**, respectively), behaved as inverse agonists and not as agonists at CB_2 receptors, i.e. as inactivators rather than activators of these receptors. This property, which we could assess only for the two most representative CB_2 ligands (i.e. for one of the compounds with, and one without, TRPV1 agonist activity), might be shared also by the other structurally similar CB_2 ligands developed in this study, and, as suggested by recent studies, might result in anti-inflammatory actions in vivo [13,14,38]. In fact, although anti-inflammatory properties have been reported more often for CB_2 receptor agonists than inverse agonists [10–12,27], it is now becoming increasingly accepted that endocannabinoids, along with their widely accepted anti-inflammatory effects, can also exert pro-inflammatory actions (e.g. by enhancing eosinophil/neutrophil and natural killer cell migration) depending on the pathological condition, experimental model and pharmacological end-point under study [39–42]. This explains why inverse agonists/antagonists and partial agonists (like Δ^9-tetrahydrocannabinol) at CB_2 receptors can also exert anti-inflammatory actions [13,14,38,39,42].

As mentioned above, apart from an entirely new chemical class of CB_2-selective ligands, an additional outcome of our successful attempts at developing "hybrid" CB_2-TRPV1 ligands, was the finding of at least one new compound still exhibiting ultra-potent activity at TRPV1 receptors and, unlike PhAR, virtually no affinity for cannabinoid CB_1/CB_2 receptors. For this compound (**15**), obtained from the introduction of a *para tert*-butyl group in the phenylacetyl moiety of PhAR,

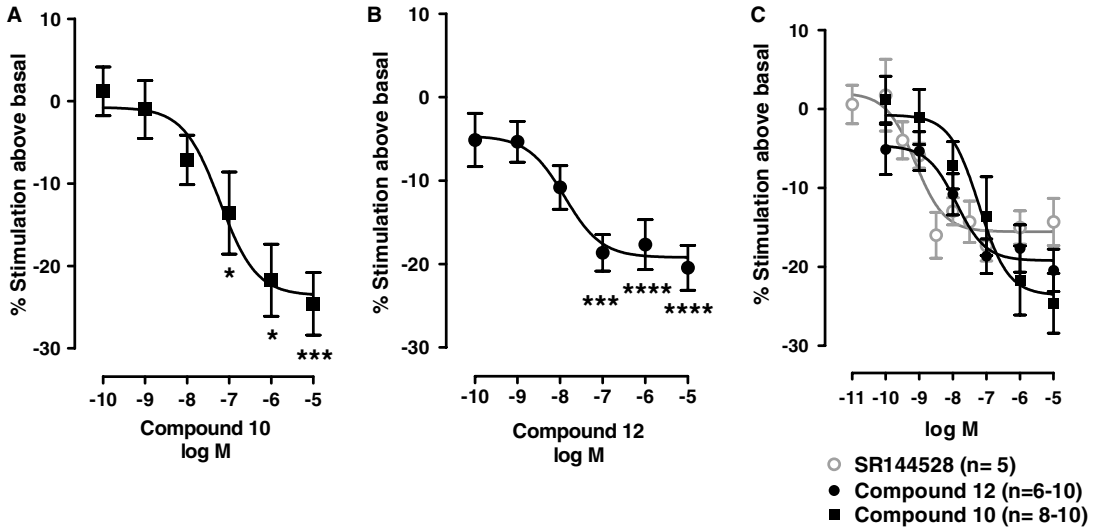


Fig. 3. Functional activity of (A) the allylamide (**10**) and (B) cyclopropylamide (**12**) of 12-phenylacetylricinoleic acid at CB_2 receptors measured as the capability of modulating GTP-γ-S binding to membranes from CHO cells stably overexpressing the human recombinant CB_2 receptor. In (C), a comparison of the two compounds with the known selective CB_2 antagonist/inverse agonist SR144528 is shown. Data are means ± S.E.M. of N = 5–10. Asterisks denote points that are statistically significantly different from zero ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$; $^{****}P < 0.001$; One-sample t-test).

one may predict similar pharmacological applications as with PhAR, i.e. the treatment of bladder hyper-reactivity [28], with little side effects after local infusion and with, perhaps, the added value of further metabolic stability to enzymatic hydrolysis due to the sterically hindered ester function.

In conclusion, our findings exemplify how modification of a pleiotropic lead like PhAR can substantially remodulate its bioactivity. PhAR was conceived as a modification of the oral analgesic and anti-inflammatory "capsaicinoid" olvanil [43], inspired by the ultra-potent TRPV1 agonist resiniferatoxin [28]. Here, by further tinkering with the structural motifs of PhAR, it was possible to: (1) temper TRPV1 potency and improve the CB_2/CB_1 selectivity ratio of this compound; (2) develop an entirely new class of ligands with inverse agonist properties at CB_2 receptors; or (3) quench PhAR affinity at cannabinoid receptors and obtain "pure" TRPV1 agonists. The first two types of compounds are original in terms of pharmacological profile or template structure, and, apart from their use as molecular probes to further our knowledge of the cannabinoid and vanilloid receptors, they also add to the growing number of anti-inflammatory leads emerging from the study on these proteins.

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